Data Evaluation Report on the acute toxicity of RPA 407213 to Sheepshead Minnow (*Cyprinodon variegatus*)
PMRA Submission Number {.....} EPA MRID Number 45385719

Data Requirement:

PMRA DATA CODE	{.....}
EPA DP Barcode	D275213
OECD Data Point	Mortality
EPA MRID	45385719
EPA Guideline	72-3

Test material: RPA 407213 **Purity:** 998 g/kg
Common name: Fenamidone (RPA 407213)
Chemical name: IUPAC: (+)-(4*S*)-4-Methyl-2-methylthio-4-phenyl-(1H)-1-phenylamino-2-imidazolin-5-one
CAS name: (S)-3, 5-dihydro-5-methyl-2-methylthio-5-phenyl-3-phenylamino 4H-imidazol-4-one
CAS No.: 161326-34-7
Synonyms: Not reported

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CITATION: Sousa, J. 1999. RPA 407213 - Acute Toxicity to Sheepshead Minnow (*Cyprinodon variegatus*) Under Flow-Through Conditions. Unpublished study performed by Springborn Laboratories, Inc., Wareham, MA. Laboratory Project No. 10566.0598.6497.505, and sponsored by Rhône-Poulenc Ag Company, Research triangle Park, NC. Sponsor Project No. 14476. Completed November 11, 1999.

EXECUTIVE SUMMARY:

The 96-h acute toxicity of fenamidone (RPA 407213) to sheepshead minnow (*Cyprinodon variegatus*) was studied under flow through conditions. Fish were exposed to a dilution water and solvent control, and 5 nominal test concentrations of RPA 407213 at 1.0, 1.7, 2.9, 4.8, and 8.0 mg a.i./L for 96 hours. The mean measured concentrations were 1.1, 1.6, 2.8, 4.8, and 7.3 mg a.i./L. The 96-h LC_{50} was 2.5 mg a.i./L and the NOAEC was 1.1 mg a.i./L, based on mortality. As a result, RPA 407213 is categorized as moderately toxic to sheepshead minnow on an acute toxicity basis.

This toxicity study is classified Core and fulfills the guideline requirement for an acute LC50 on an estuarine fish with technical fenamidone.

Results Synopsis

Test Organism Size/Age (mean Weight or Length): 0.41g and 2.6 cm
Test Type (Flowthrough, Static, Static Renewal): Flow-through
LC_{50}: 2.5 mg a.i./L 95% C.I.: 2.2-2.9 mg a.i./L
NOAEC: 1.1 mg a.i./L Probit Slope: N/A
EC_{50}: N/A Endpoint(s) Affected: Mortality and sublethal effects such as lethargy and loss of equilibrium

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: The study was conducted in accordance with the Standard Evaluation Procedure, Acute Toxicity Test for estuarine and marine organisms (EPA-540/9-85-006, 72-3).

Deviations included the following:

1) The mean weight of the fish at initiation was (0.41 g), which is slightly lower than EPA guidelines (0.5 -5.0g).

2) The aquarium size and fill volume were smaller (30 x 15 x 20 cm and 6.8 L, respectively) than required by EPA guidelines (30 x 60 x 30 cm and 15-30 L of solution).

COMPLIANCE: Signed and dated GLP, Quality Assurance and Confidentiality statements were provided.

A. MATERIALS:

1. Test Material Fenamidone (RPA 407213)

Description: White Powder

Lot No./Batch No. : CDR 9705

Purity: 998 g/kg

Stability of Compound Under Test Conditions: Test concentrations were stable at the end of the study period. Measured concentrations after 96 hours ranged from 86-94% of concentrations measured at test initiation.
(OECD requires water solubility, stability in water and light, pKa, Pow, vapor pressure of test compound)

Storage conditions of test chemicals: The test substance was stored at room temperature in the dark.

2. Test organism:

Species: Sheepshead minnow (*Cyprinodon variegatus*)
OECD allows choice of species at discretion of testing laboratory.
Age at test initiation: Not reported
Weight at study initiation: 0.41 g (0.16-0.84)
EPA requires: mean 0.5 - 5 g
Length at study initiation: 26mm (19-36 mm)

Source: Aquatic Biosystems (Ft. Collins, CO)

B. STUDY DESIGN:

1. Experimental Conditions

a) Range-finding Study

Range Finding Study: One static and two flow-through range-finding studies with RPA407213 were conducted in order to estimate the nominal concentration range for the definitive study. The 48-hour static test exposed sheepshead minnow to nominal concentrations of 1.0, 10.0, and 100 mg a.i./L. Response to these conditions was compared to a dilution water control. Following 48 hours, 100% mortality was observed in the 10 and 100 mg a.i./L test concentrations, and no mortality or sublethal effects were observed in the 1.0 mg a.i./L test concentration. In the first flow-through test, sheepshead minnows were exposed to nominal concentrations of 0.13, 0.22, 0.36, 0.60 and 1.0 mg a.i./L. Following 96 hours, no mortality or sublethal effects were observed. In the second flow-through test, sheepshead minnows were exposed to nominal concentrations of 1.0, 1.7, 2.9, 4.8 and 8.0 mg a.i./L. Following 96 hours, 100% mortality was observed in the 4.8 and 8.0 mg a.i./L test concentrations, 40% mortality and 100%sublethal effects were observed in the 2.9 mg a.i./L concentration, and no mortality or sublethal effects were observed in the remaining concentrations.

b) Definitive Study

Table 1 . Experimental Parameters

Parameter	Details	Remarks Criteria
Acclimation: period: conditions: (same as test or not)	 14 days Same as test	
Feeding: Health: (any mortality observed)	Not fed 48-hours prior to test initiation; not fed during exposure 0% mortality 48 hours prior to test initiation	*EPA requires: minimum 14 days; no feeding during test OECD requires minimum of 12 days.*
Duration of the test	96-hour	*(EPA/OECD requires: 96 hour)*

Parameter	Details	Remarks Criteria
Test condition static/flow through Type of dilution system- for flow through method. Renewal rate for static renewal	 Flow-through Intermittent flow proportional diluter 8.2 solution volume replacements/day	 *(EPA requires: Must provide reproducible supply of toxicant)* *(EPA requires: Consistent flow rate of 5-10 vol/24 hours, meter systems calibrated before study and checked twice daily during test period*
Aeration, if any	None	*(EPA requires: no aeration; OECD permits aeration)*
Test vessel Material: (glass/stainless steel) Size: Fill volume:	 Glass aquaria 30 x 15 x 20 cm 6.8 L	 *EPA requires: Size 19 L (5 gal) or 30 x 60 x 30 cm* *Fill volume: 15-30 L of solution*
Source of dilution water Quality:	Natural filtered seawater	*(EPA 1975; Soft reconstituted water or water from a natural source, **not** dechlorinated tap water); OECD permits dechlorinated tap water.*

Parameter	Details	Remarks Criteria
Water parameters: Hardness pH Dissolved oxygen Total Organic carbon Particulate Matter Metals Pesticides Chlorine Temperature Salinity Intervals of water quality measurement	 Not reported 7.7-8.2 60 - 93% saturation (4.4-6.8 mg/L) <2 mg/L <9.0 mg/L Below detection limit Below detection limit <0.2 mg/L 22°C 30-31% DO, pH, salinity, and temperature were measured every 24 hours.	Analysis pp 51-53 *(EPA hardness: 40 - 48 mg as $CaCO_3$/L; OECD allows 10 -250 mg as $CaCO_3$/L)* *(EPA pH: 7.2 - 7.6; 8.0-8.3 for marine-stenohaline fishes, 7.7-8.0 for estuarine-euryhaline fishes, monthly range < 0.8); OECD allows pH 6.0 - 8.5* (EPA Dissolved Oxygen: *Static: ≥ 60% during 1st 48 hrs and ≥ 40% during 2nd 48 hrs, flow-through: ≥ 60%); OECD requires at least 80% saturation value.* *(EPA temperture: estuarine/marine: 22 ± 1 °C OECD requires 21 - 25°C for bluegill and 13 - 17°C for rainbow trout* *(EPA salinity: 30-34 ‰ (parts per thousand) salinity, weekly range < 6 ‰)* *(EPA water quality: measured at beginning of test and every 48 hours)*
Number of replicates/groups: control: solvent control: treated ones:	 2 2 2	 *(EPA/OECD requires: Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series)*
Number of organisms per replicate /groups: control: solvent control: treated ones:	 10 10 10	 *(EPA: ≥ 10/concentration); OECD requires at least 7 fish/concentration*

Parameter	Details	Remarks Criteria
Biomass loading rate	0.073 g/L	*Static: ≤ 0.8 g/L at ≤ 17°C, ≤ 0.5 g/L at > 17°C; flow-through: ≤ 1 g/L/day; OECD requires maximum of 1 g fish/L for static and semi-static with higher rates accepted for flow-through*
Test concentrations: nominal: measured:	 1.0, 1.7, 2.9, 4.8, 8.0 mg a.i./L 1.1, 1.6, 2.8, 4.8, 7.3 mg a.i./L	
Solvent (type, percentage, if used)	Dimethlyformamide, 0.097 ml/L	*EPA requires: Not to exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-through tests; OECD requires solvent , exceed 100 mg/L.*
Lighting	16:8	*(EPA requires: 16 hours light/8 hours dark); OECD requires 12 -16 hours photoperiod.*
Feeding	Not fed 48-hour prior to study or during exposure	*EPA/OECD requires: No feeding during the study*
Recovery of chemical Level of Quantitation Level of Detection	91-110% 4.24 µg a.i./L Not reported	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	N/A	

2. **Observations:**

Table 2: Observations

Criteria	Details	Remarks/Criteria
Parameters measured including the sublethal effects/toxicity symptoms	Mortality and sublethal effects	
Observation intervals	Mortality and sublethal effects were evaluated at 24, 48, 72 and 96 hours of exposure	*(EPA/OECD requires: minimally every 24 hours)*
Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS and DISCUSSION:

A. MORTALITY:

Following 48 hours, 100% mortality was observed in the 4.8 and 7.3 mg a.i./L test concentrations. Following 96-hours, 15% and 50% mortality was observed in the 1.6 and 2.8 mg a.i./L test concentrations, respectively. Sublethal effects were observed in 100% of the surviving sheepshead minnows in the 2.8 mg a.i./L test concentration. No mortality or sublethal effects were observed in the 1.1 mg a.i./L test concentration.

Table 3: Effect of RPA 407213 on mortality of Sheepshead minnow (*Cyprinodon variegatus*).

Treatment nominal and (measured) concentrations (mg a.i./L)	No. of fish at start of study	Observation period							
		Day 1		Day 2		Day 3		Day 4	
		No. Dead	Per Cent Mortality	No. Dead	Per Cent Mortality	No. Dead	Per Cent Mortality	No. Dead	Per Cent Mortality
Control (dilution water only)	20	0	0	0	0	0	0	0	0
Solvent control	20	0	0	0	0	0	0	0	0
1.0 (1.1)	20	0	0	0	0	0	0	0	0
1.7 (1.6)	20	0	0	0	0	3	15	3	15
2.9 (2.8)	20	2	10	5	25	7	35	10	50
4.8 (4.8)	20	15	75	19	95	20	100	20	100

Treatment nominal and (measured) concentrations (mg a.i./L)	No. of fish at start of study	Observation period							
		Day 1		Day 2		Day 3		Day 4	
		No. Dead	Per Cent Mortality	No. Dead	Per Cent Mortality	No. Dead	Per Cent Mortality	No. Dead	Per Cent Mortality
8.0 (7.3)	20	18	90	20	100	20	100	20	100
NOAEC	1.1 mg a.i./L								
LC_{50}	2.5 mg a.i./L								
Positive control, if used mortality: LC_{50}:	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

B. NON-LETHAL TOXICITY ENDPOINTS:

Sublethal effects were noted for all surviving fish exposed to the 2.8 mg a.i./L test concentration, including partial or complete loss of equilibrium. No sublethal effects were described for fish exposed to concentrations of 1.1 or 1.6 mg a.i./L.

Table 4. Sub-lethal effect of RPA 407213 on Sheepshead minnow (*Cyprinodon variegatus*).

Treatment nominal and (measured) concentrations (mg a.i./L)	Observation period			
	endpoint at Day 1	endpoint at Day 2	endpoint at Day 3	endpoint at Day 4
	% affected	% affected	% affected	% affected
Control (dilution water only)	0	0	0	0
Solvent control	0	0	0	0
1.0 (1.1)	0	0	0	0
1.7 (1.6)	0	0	0	0
2.9 (2.8)	55	100	100	100
4.8 (4.8)	100	100	N/A	N/A
8.0 (7.3)	100	N/A	N/A	N/A
NOAEC	1.6 mg a.i./L			

Treatment nominal and (measured) concentrations (mg a.i./L)	Observation period			
	endpoint at Day 1	endpoint at Day 2	endpoint at Day 3	endpoint at Day 4
	% affected	% affected	% affected	% affected
LOAEC	2.8 mg a.i./L			
EC_{50}	Not reported			
Positive control, if used % sublethal effect: EC_{50}:	N/A	N/A	N/A	N/A

C. REPORTED STATISTICS:

The LC_{50} was determined by moving average angle analysis.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: The LC_{50} and 95% confidence interval was estimated using the moving average angle method via TOXANAL software. The NOAEC was determined visually based on mortality. There was no mortality in any of the control groups.

LC_{50}: 2.5 mg a.i./L 95% C.I.: 2.2-2.9 mg a.i./L
NOAEC: 1.1 mg a.i./L

E. STUDY DEFICIENCIES:

The fish used in this experiment were smaller and the aquarium size and fill volume were less than US EPA recommends for toxicity testing with estuarine/marine fish (EPA-540/9-85-006, 72-3). However these deviations did not impact the acceptability of the study.

F. REVIEWER'S COMMENTS:

The reviewer's conclusions were identical to those of the study authors. The 96-h LC_{50} was 2.5 mg a.i./L and the NOAEC was 1.1 mg a.i./L, based on mortality. As a result, RPA 407213 is categorized as moderately toxic to sheepshead minnow on an acute toxicity basis.

G. CONCLUSIONS: There was no mortality during the study and no sign of sublethal toxicity.

Complete mortality was observed for sheepshead minnow (*Cyprinodon variegatus*) in the 4.8 and 7.3 mg a.i./L test

concentrations after 96 hours. The 1.7 and 2.9 mg a.i./L test concentrations elicited 15 and 50% mortality. Sublethal effects were observed for all surviving fish in the 2.8 mg a.i./L test concentration, and included partial or complete loss of equilibrium. The LC_{50} was 2.5 mg a.i./L and the NOAEC was 1.1 mg a.i./L (based on sublethal effects). As a result, RPA 407213 is classified as moderately toxic to sheepshead minnow *(Cyprinodon variegatus)* according to the classification system of the U.S. EPA.

This toxicity study is classified Core.

LC_{50}: 2.5 mg a.i./L 95% C.I.: 2.2-2.9 mg a.i./L
NOAEC: 1.1 mg a.i./L

III. REFERENCES:

APHA, AWWA, WPCF. 1992. Standard Methods for the Examination of Water and Wastewater. 18th Edition, Washington, DC, 2168 pp.

ASTM. 1997. Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. Standard E729-88a, 25 pp. American Society for Testing and Materials, 1916 Race Street, Philadelphia, PA. 19103.

Mount, D.I. and W.A. Brungs. 1967. A Simplified Dosing Apparatus for Fish Toxicological Studies. Water Research 1: 21-29.

Sprague, J.B. 1969. Measurement of Pollutant Toxicity to Fish. 1. Bioassay Methods for Acute Toxicity. Water Research 3: 793-821.

U.S. EPA. 40 CFR, Part 160. Federal Insecticide, Fungicide and Rodenticide Act. Goo Laboratory Practices Standards; Final Rule. Office of the Federal Register, National Archives and Records Administration. U.S. Government Printing Office, Washington, D.C.

U.S. EPA. 1975. Methods for Acute Toxicity Test with Fish, Macroinvertebrates and Amphibians. Ecological Research Series (EPA-660/3-75-009). U.S. Environmental Protection Agency, Washington, D.C., 61 pp.

U.S. EPA. 1982. Office of Pesticide Programs. Pesticide Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms. EPA-540/9-85-024. October 1982. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1985. Office of Pesticide Programs. Standard Evaluation Procedure for Acute Toxicity Test for Freshwater Fish. EPA-540/9-85-006. June 1985. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 1994. Pesticide Reregistration rejection Rate Analysis. Ecological Effects. EPA 738-R-035, U.S. Environmental Protection Agency, Washington, D.C.